

CLAIMS

1. Method for the preparation of crosslinked enzyme aggregates, comprising enzyme molecules being associated with an enzyme activity-enhancing agent, the enzyme activity-enhancing agent interacting with the said enzyme molecules, comprising the steps of:

A - providing a plurality of enzyme molecules,

B - aggregating the enzyme molecules in a liquid medium, by means of addition of a precipitating agent,

C - crosslinking the aggregated enzyme molecules to one another by providing a crosslinking agent in the liquid medium,

the method comprising, during or between steps A, B or C, the step of adding an enzyme activity-enhancing agent and allowing the enzyme molecules to become and to remain associated with the enzyme activity-enhancing agent.

2. Method according to claim 1, wherein the enzyme molecules comprise cofactor dependent enzyme molecules.

3. Method according to claim 1 or 2, wherein the enzyme activity-enhancing agent comprises a cofactor.

4. Method according to claim 3, wherein the cofactor is chosen from the group of nicotinamide cofactors, comprising NADH, NAD⁺, NADPH, NADP⁺.

5. Method according to claim 3, wherein the cofactor is an analogue of the nicotinamide cofactor.

6. Method according to any of the preceding claims, wherein the enzyme is an alcohol dehydrogenase.

7. Method according to claim 5, wherein the alcohol dehydrogenase is derived from *Lactobacillus kefir* or horse liver.

8. Method according to any of the preceding claims, wherein the crosslinking agent being prepared by combining a first and a second compound each having at least two reactive groups, the reactive group

of the first compound being primary amino groups, the reactive groups of the second compound being aldehyde groups.

9. Method according to any of the preceding claims 2-8, wherein at least two cofactor dependent enzymes are provided in step A) and wherein at least one, preferably more than one, enzyme activity-enhancing agents are added during or between steps A), B) or C).

10. Method according to any of the preceding claims, wherein steps B) and C) are performed simultaneously.

11. Method according to any of the preceding claims, further comprising the steps of:

D) performing an enzyme catalysed reaction in a liquid medium in the presence of crosslinked enzyme aggregates, obtained in step C), and E) recycling the crosslinked enzyme aggregates of step D).

12. Method according to claim 11, wherein steps D) and E) are combined and wherein, in step D), at least two enzyme catalysed reactions are performed and wherein the crosslinked enzyme aggregates comprises at least two cofactor dependent enzymes, the first enzyme being active in catalysing the first reaction when associated with the cofactor in oxidised state, the second enzyme being active in catalysing the second reaction when associated with the said cofactor in reduced state.

13. Method according to any of the preceding claims, wherein enhanced stabilisation of the enzyme system allow for recycle of the CLEA.

14. Crosslinked enzyme aggregates of enzymes, obtainable according to the method according to any of the preceding claims.

15. Crosslinked enzyme aggregates of enzymes comprising an enzyme activity-enhancing agent associated therewith.